MRD as prognostic marker in CLL

Jan Philippé
Therapeutic strategies in CLL

<table>
<thead>
<tr>
<th>Physical fitness</th>
<th>Comorbidity</th>
<th>Life expectancy</th>
<th>Principle of action</th>
<th>Therapeutic goal and treatment options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functionally independent</td>
<td>None or mild</td>
<td>Normal (reduced by malignant disease only)</td>
<td>‘Go go’</td>
<td>Therapy aimed at prolonging survival, such as FCR</td>
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<tr>
<td>Reduced physical fitness</td>
<td>≥1 comorbidity</td>
<td>Intermediate (impaired by malignancy, comorbidity and/or unfit state of health)</td>
<td>‘Slow go’</td>
<td>Therapy aimed at achieving optimal symptom control, such as monotherapies (chlorambucil or bendamustine) or dose-reduced combinations</td>
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<tr>
<td>Severely handicapped</td>
<td>Multiple or severe comorbidities</td>
<td>Short (severely reduced by comorbidities and/or frail state of health)</td>
<td>‘No go’</td>
<td>Best supportive palliative care</td>
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</tbody>
</table>

When to treat?

If CLL disease is **progressive**
No curative therapy

Alkylating agents, McAbies, Corticoids

However,
- Except allogeneic BM transplantation (6 in UZ Gent) (long term remission)
- New therapeutics with the potential to reach complete molecular remission
  - Anti-CD20 mAb (Rituximab, Ofatumumab, …)
  - Anti-CD23 mAb (Lumiliximab)
  - Anti-CD40 (Dacetuzumab)
  - Anti-CD52 (Alemtuzumab)
  - Bcl-2 family of inhibitors (Oblimersen, Obatoclax)
  - Immunomodulating agents (Lenalidomide (anti-TNF, anti-angiogenic, anti-T<sub>act</sub>, …))
  - Protein kinase inh (flavopiridol)

Garcia-Escobar et al.
Crit Rev in Oncol/Hematol 2010
How to monitor CLL?

- Without progression:
  - ‘Watch and wait’ → cell count is sufficient
- In ‘slow go’ → symptom control
- If CR/molRem is attempted → MRD analyses
  - Quantitative monitoring with RQ-PCR
  - Quantitative monitoring with flow cytometry
What is the ideal MRD assay?

- CLL-specific
- High sensitivity, also in the presence of normal B cells
- Simplicity
- Possibility for quantification
Molecular methods used for follow-up in CLL

**Consensus PCR**
Consensus primers against the framework regions and the \( J_H \) region
- drawbacks: limited sensitivity (1%)
- no quantification

**Clone-specific PCR (nested)**
1\(^{st} \) step consensus IgH PCR
2\(^{nd} \) step with 1 or 2 allele-specific primers (CDR2 and CDR3)
- advantage: high sensitivity (\( 10^{-6} \))
- drawback: need for individual sequencing
- no quantification

**Clone-specific real-time quantitative PCR (RQ-PCR)**
Combination of patient-specific primers (CDR2 and CDR3) and a consensus probe (FR3)
- advantage: quantification
- drawback: labor intensive (sequencing, primer-probe test)
- decreased sensitivity (\( 10^{-4} \))
International standardized approach for FC residual disease monitoring in CLL (A. Rawstron et al. Leukemia 2007)

- 50 CLL specific antibody combinations were tested for 4-color FC

- **Protocol:**
  - Start with $10^6$ cells, use whole blood or BM
  - NH$_4$Cl lysis (10’) followed by 2 washings
  - Staining
  - FACSLyse (according to the manufacturer’s protocol)
  - Analysis performed on CD19+ gated B cells
International standardized approach for FC Residual disease monitoring in CLL (A. Rawstron et al. Leukemia 2007)

3 combinations turned out to have low inter-laboratory variation and false detection rates

- FITC / PE / PerCP / APC
- CD20 / CD38 / CD19 / CD5
- CD81 / CD22 / CD19 / CD5
- CD43 / CD79b / CD19 / CD5
Figure 3  Use of a consensus protocol improves precision, consistency and specificity for detection of MRD by operators who are experienced in flow cytometric analysis of CLL.
Standardized MRD flow and ASO IgH RQ-PCR for MRD quantification in CLL (S Böttcher et al, Leukemia 2009)

- Protocol based upon previous papers
  - 200 µL of whole blood (2x10^6 cells) are washed
  - Incubation with rabbit serum
  - Stained with 4 colors
  - + FACSLyse & 2 washings
  - Use of isotype controls

- FITC / PE / PerCP / APC
- CD20 / CD5 / CD19 / CD43
- CD81 / CD22 / CD19 / CD5
- CD79b / CD20 / CD19 / CD5
- K / λ / CD19 / CD5
Standardized MRD flow and ASO IgH RQ-PCR for MRD quantification in CLL (S Böttcher et al, Leukemia 2009)

Figure 1  Correlation of minimal residual disease (MRD) results obtained by MRD flow and by allele-specific oligonucleotide primer IgH real-time quantitative (RQ)-PCR (ASO IgH RQ-PCR) according to treatment arm (+< qr: positive, outside quantitative range). The numbers in the graphs give sample numbers according to MRD flow and RQ-PCR results. Concordantly, positive samples show a significant quantitative correlation in both treatment arms: \( r = 0.95 \), \( P < 0.0001 \) for each treatment arm.
ESCCA 2010: S. Böttcher (Kiel)

- Euroflow based approach

<table>
<thead>
<tr>
<th></th>
<th>FITC</th>
<th>PE</th>
<th>PerCP Cy5.5</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>APCH7</th>
<th>V450</th>
<th>V500</th>
</tr>
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<tbody>
<tr>
<td>IgM</td>
<td>CD5</td>
<td>CD79b</td>
<td>CD19</td>
<td>CD200</td>
<td>------</td>
<td>CD20</td>
<td>CD45</td>
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Follow-up sample from a patient, 1 year post allotransplant
Follow-up sample from a patient, 1 year post allotransplant
Follow-up sample from a patient, 1 year post allotransplant
Impact of MRD status 12 months after allotransplant
N=43, event-free after 12 m

Percent relapsed

+12 MRD- (32)

+12 MRD+ (11)

HR 0.037 (0.008-0.18); p <0.0001

Months from SCT

Boettcher et al
Blood Rev 2011
Thank you

Questions?